

CULTIVATION OF EIMERIA DISPERSA
IN BOBWHITE QUAIL EMBRYOS

An abstract of a Thesis by
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The problem. To redescribe the sporulated oocysts of Eimeria dispersa from the Bobwhite quail (Colinus virginianus), to study the endogenous development of E. dispersa in Bobwhite quail embryos, and to examine the cross-transmission properties of this species for turkeys, chickens, and Japanese quail.

Procedure. Oocysts, isolated from fecal samples of adult Bobwhites, were sporulated in an aqueous solution containing 2.5% potassium dichromate. Oocysts used for redescription were isolated by sugar flotation and studied with the aid of a compound microscope equipped with achromatic objectives. Oocysts used as a source of inoculum were isolated by sugar flotation, washed, placed in a tissue grinder, and crushed. Sporocysts, freed by crushing, were isolated by centrifugation and placed in an excystation medium containing 5.0% chicken bile and 0.25% trypsin in Tyrode's solution. Between 1,000 and 44,000 sporozoites were inoculated into the allantoic cavity of 10- to 12-day-old embryos with the aid of a tuberculin syringe. The allantoic fluid and chorioallantoic membranes were removed from inoculated eggs at 12-hour intervals from $\frac{1}{2}$ through 10 days after inoculation. The chorioallantoic membranes were fixed in Zenker's fluid, stained with hematoxylin and eosin, and studied with bright-field and phase-contrast microscopy. Oocysts were harvested from the allantoic fluid, sporulated, and inoculated into a 3-month-old Bobwhite in order to demonstrate infectivity. In order to examine the cross-transmission properties of E. dispersa, two 2-month-old domestic turkeys, two 2-week-old White Leghorn chickens, and two 2-week-old Japanese quail were inoculated with oocysts of this species.

Findings. Subspherical to ovoid oocysts were found in the feces 4 to $4\frac{1}{2}$ days after inoculation. Sporulated oocysts averaged 22.4 by 18.5 micrometers. Sporocysts were asymmetrically ellipsoidal and averaged 14.1 by 6.7 micrometers. Extended free sporozoites averaged 13.8 by 2.7 micrometers. All tissue stages developed within epithelial cells of the chorioallantoic membrane. Intracellular sporozoites were present from day 2 until day $9\frac{1}{2}$ post-inoculation. Fixed

intracellular sporozoites averaged 7.8 by 4.0 micrometers. These developed into sporozoite-shaped schizonts which were observed between 2 and 9½ days after inoculation, and averaged 10.0 by 3.7 micrometers. Mature large schizonts, probably representing those of a first generation, developed from the sporozoite-shaped schizonts and were observed between 2½ and 10 days post-inoculation. These large schizonts averaged 16.7 by 12.4 micrometers and contained an average of 24 merozoites. Smaller schizonts, probably those of a second generation, were observed between 4 and 10 days after inoculation and contained an average of 12 merozoites. These small schizonts averaged 9.9 by 7.9 micrometers. Mature microgametocytes averaging 18.7 by 14.9 micrometers were observed on days 4 through 10. Mature macrogametes averaging 19.2 by 15.0 micrometers were observed on days 8 through 10 post-inoculation. Oocysts developing in epithelial cells of the chorioallantoic membrane were observed between 8 and 9½ days after inoculation. Non-sporulated oocysts were found in the allantoic fluid at 9 and 9½ days post-inoculation and averaged 19.8 by 17.0 micrometers. The chorioallantoic membranes showed no macroscopic lesions. Slight hyperemia and hemorrhaging were observed in scattered areas near large blood vessels during schizogony and gametogony. In adult birds, the prepatent period was 4 to 4½ days, and oocysts were found in large numbers until 15 days post-inoculation when only a few oocysts were present in fecal samples. When embryo-derived oocysts were inoculated into a coccidia-free juvenile, the prepatent period was delayed until 6 days post-inoculation. Attempts to transmit E. dispersa to domestic turkeys, White Leghorn chickens, and Coturnix quail were unsuccessful.

Conclusions. The sporulated oocysts of E. dispersa from the Bobwhite quail are redescribed, and their endogenous development in quail embryos is reported. In embryos, the life cycle appears to be delayed beyond that observed in mature birds. When embryo-derived oocysts are reinoculated into young Bobwhite quail, the prepatent period is also delayed beyond that observed in birds inoculated with oocysts derived from natural sources. As in naturally infected birds, 2 generations of schizonts appear to be present in the life cycle in embryos, and the gametocyte stages appear to be similar to those reported for this species. E. dispersa was found to be relatively non-pathogenic in adult Bobwhites, but markedly pathogenic in young Bobwhites 2 weeks of age and younger. Attempts to infect turkeys, chickens, and Japanese quail with sporulated oocysts of E. dispersa isolated from adult Bobwhite quail were unsuccessful, indicating that E. dispersa may be host specific solely for Bobwhite quail.

Recommendations. Future research concerning the cultivation of E. dispersa should provide more detailed light microscope descriptions of the endogenous stages in the adult Bobwhite quail and its embryos. Also, attempts should be directed toward the description and cultivation of the endogenous stages of this species in cell cultures. The fine-structure of these stages, both in vivo and in vitro, should also be studied. The phenomenon of a lengthened prepatent period in Bobwhites inoculated with embryo-derived oocysts, as opposed to those given naturally-derived oocysts, should also be investigated.

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INTRODUCTION AND REVIEW OF THE LITERATURE

Most parasites of the genus Eimeria have pronounced host and site specificity and typically undergo endogenous development in cells of the intestinal tract (Long, 1965). However, studies by Long (1965, 1966, 1969, 1970, 1971, 1973), Baldelli et al. (1968, cited by Doran, 1973), Ryley (1968), Shibalova (1968, 1969, 1970), Jeffers and Wagenbach (1969, 1970), and Fitzgerald (1970) have shown that certain species of coccidia grow extremely well in avian embryos. Other studies (Doran, 1970, 1973) have shown that in vitro cell culture of avian and mammalian cells is also a successful means of cultivating coccidia. Both of these methods of cultivating coccidia provide an opportunity to study the parasites outside of the host where compounding factors such as host resistance, etc. can be held to a minimum. Growing coccidia in avian embryos has the advantage of providing large numbers of oocysts (Long, 1969), while growing coccidia in cell culture has the advantage of providing host cells that can be observed continuously during the endogenous cycle of the parasite. Also, many species of coccidia can be grown in a wide variety of avian and mammalian primary cells and mammalian cell lines using the latter method (Strout et al., 1969).

Studies concerned with the coccidia from Bobwhite quail are few in number, and no work has been reported

concerning the cultivation of coccidia in Bobwhite quail embryos.

Since little information is available about the coccidia of the Bobwhite, further study seems appropriate. In the present study, Eimeria dispersa from the Bobwhite quail is redescribed, and the cultivation of this species in Bobwhite quail embryos is demonstrated.

The first coccidian species to be described from the Bobwhite quail was Eimeria dispersa by Tyzzer (1929). Venard (1933) stated that he found E. dispersa, as well as E. tenella and E. acervulina oocysts in fecal samples of naturally infected Bobwhites. Levine (1953) stated that the only properly described species occurring in the Bobwhite quail was E. dispersa. Hawkins (1952) and Pellérdy (1965) also considered E. dispersa as the only valid species of coccidia known to parasitize the Bobwhite. Waggoner (1967) reported another species of coccidia from the Bobwhite which he designated as an Eimeria sp. and suggested, as a result of a personal communication with Battle, that an additional Eimeria sp. also probably exists.

Detailed descriptions concerning the biology of the coccidian parasites reported from the Bobwhite quail appear to be limited to those made by Tyzzer (1929) and by Waggoner (1967).

Tyzzer (1929) discovered that the endogenous cycle of

E. dispersa occurred only within epithelial cells of the villi throughout the length of the small intestine, with the heaviest infections occurring in the upper regions of the intestine. The developing parasites were observed to lie either above, below, or along side the nuclei of the epithelial cells. After entering the epithelial cells, the sporozoites were not immediately transformed into trophozoites, but persisted as elongate forms until they attained considerable size. Schizonts of this species were found by Tyzzer to be of 2 distinct types. The first type varied greatly in size and produced numerous short merozoites. The second type was usually smaller than the first, and produced fewer but larger sausage-shaped merozoites which were arranged with their long axes nearly parallel. Microgametocytes and macrogametes were approximately the same size and assumed positions within the epithelial cells which were similar to those observed for the schizonts. Oocysts were first recovered in the feces between 4 and 5 days after inoculation.

Tyzzer (1929) also found heavily infected mature Bobwhite quail to remain in relatively good condition and to maintain their normal weight during infection. Heavy infections in very young quail, however, were usually fatal. Juvenile Bobwhites exhibited severe diarrhea and retardation of growth when heavily infected with E. dispersa. Chronic

cases were found to result in the discharge of oocysts for several months while the birds became thin and underweight.

In cross-transmission studies, Tyzzer (1929) stated that he successfully transmitted E. dispersa from the Bobwhite quail to chickens and to turkeys, but he could not infect pheasants with E. dispersa from the same source. In other studies, Patterson (1933) and Venard (1933) were unable to infect chickens with E. dispersa from the Bobwhite, and Hawkins (1952) found it impossible to infect chickens or pheasants with E. dispersa from the Bobwhite. However, Hawkins (1952) successfully produced E. dispersa infections in the turkey and Hungarian partridge with inoculum obtained from turkeys.

Waggoner (1967) reported an Eimeria species from Bobwhite quail which primarily parasitizes the upper small intestine, but which in severe infections may extend from the duodenum to the caecum. All developmental stages were observed by Waggoner (1967) to lie above the epithelial cell nuclei within the villi of the intestine. The endogenous cycle contained 2 schizogonous generations, with schizonts of the first generation appearing smaller than those of the second, in most cases; some second-generation schizonts were similar in size to those of the first generation. The smaller schizonts contained fewer merozoites than did the larger schizonts, but merozoites of both schizonts were

approximately the same size. Within the epithelial cells, both microgametocytes and macrogametes appeared to be of similar size. Oocysts were first observed in the feces at about 3 days post-inoculation.

Waggoner (1967) also found that during the course of infection with this Eimeria species, that the intestines of the hosts were flaccid and the contents watery. Some hemorrhaging was also observed. In 11-day-old Bobwhites, severe infections caused growth depression, and inoculation with 0.5 million oocysts resulted in a mortality rate of 15%. Waggoner (1967) failed to transmit Eimeria sp. to chickens, turkeys, and Coturnix quail.

In another Eimeria species, which Battle described to Waggoner (1967) in a personal communication, the parasites occurred primarily within epithelial cells of the villi lining the jejunum. There appeared to be 2 generations of schizonts associated with this species also, with schizonts of both generations being approximately equal in size and containing merozoites which were also equal in size. Microgametocytes and macrogametes were also similar to each other in size. The schizonts were located above the epithelial cell nuclei while the gametocytes were normally located below the nuclei of the epithelial cells. The prepatent period was $3\frac{1}{4}$ days, and this species was shown to be more pathogenic than that studied by Waggoner (1967).

Regarding the cultivation of avian coccidia in avian embryos, Long (1965) was the first to report the growth of a coccidian in eggs and found that E. tenella would develop within the chorioallantoic membrane of chick embryos following inoculation of sporozoites into the allantoic cavity. The infection which Long observed was confined to the chorioallantoic membrane and did not occur in the embryo itself. Long found schizonts in histological sections of chorioallantoic membranes 4 to 9 days after inoculation, and gametocytes and oocysts between 7 and 11 days post-inoculation. Numerous merozoites were observed free in the allantoic cavity on days 6 and 7 while oocysts were found free in the allantois on days 7 through 11. Long also reported a slight delay in the length of the life cycle of E. tenella grown in embryos, apparently resulting from an extended period of schizogony.

Long (1966) attempted to produce embryonic infections by introducing either sporulated oocysts, sporocysts, sporozoites, or merozoites of E. tenella into the allantoic cavity of chick embryos. Oocysts failed to produce infections, and when large numbers of sporocysts were inoculated, only slight infections could be detected. Sporozoites inoculated into the allantoic cavity, however, caused extensive infection of the chorioallantoic membrane, and oocysts were recovered from the allantois 7 to 10 days post-inoculation.

Oocysts obtained from the allantoic cavity sporulated normally and produced characteristic infections when given orally to 2-week-old chickens. When large quantities of second-generation merozoites from adult birds were injected into the allantoic cavity of chick embryos, numerous oocysts could be recovered 2 to 3 days after inoculation.

Ryley (1968), who studied the effects of chemotherapeutic agents on the growth of E. tenella in the chorioallantoic membranes of chicken eggs, demonstrated mature schizonts 4 to 5 days after the inoculation of sporozoites; gametocytes were observed on days 7 through 9. Ryley (1968) found that certain drugs could protect chick embryos from death due to infection with E. tenella, but that some of these drugs did not give comparable protection in chickens infected with E. tenella.

Baldelli et al. (1968), Shibalova (1968, 1969, 1970), and Shibalova et al. (1969) were also successful in infecting chick embryos with E. tenella, and all obtained results similar to those reported by Long (1965).

Jeffers and Wagenbach (1969) also successfully infected chick embryos with E. tenella. They administered 10 different dosage levels of sporozoites, ranging in concentration from 1×10^4 to 1×10^6 sporozoites per egg, into the allantoic cavity of 10-day-old embryos. Those embryos receiving 1×10^4 sporozoites showed the lowest mortality

rate, and this rate increased progressively as the dosage level of sporozoites increased. It was also shown that there was a greater mortality rate at all dosage levels in female embryos, compared to male embryos which showed a lower rate for all dosage levels.

Jeffers and Wagenbach (1970) discovered that infections with E. tenella did not change the weight of chicken eggs or the weight of the hatched chicks. Chicks hatched from eggs inoculated with sporozoites were able to withstand a challenge dose of E. tenella oocysts better than chicks hatched from noninoculated eggs which showed a higher mortality rate when challenged.

Long (1970) found a direct relationship between the quantity of sporozoites introduced into the allantoic cavity and mortality caused by hemorrhage. Long (1970) also found that incubating sporozoites at 41° C before inoculation, stimulated schizogony, and resulted in greater mortality of the embryos in a shorter period of time. Conversely, incubating sporozoites at temperatures of 38° and 39° C prior to inoculation, retarded the rate of schizogony, and lowered the rate of mortality in embryos.

Infections in the liver and bile ducts of chick embryos, inoculated intravenously, have also met with some success (Long, 1971). Long observed schizogonous and gametogonous stages of E. tenella in epithelial cells of the

bile ducts and in liver parenchymal cells of embryos inoculated intravenously. Oocysts were also seen in both of these cell types.

Long (1973) compared the development of an embryo-adapted strain of E. tenella in the chorioallantoic membrane with that of the same strain grown in the intestine of the chicken. He found that successive embryo passages reduced the pathogenicity of this parasite for both embryos and chickens. Cultures of oocysts passaged 41 times in embryos were able to regain their pathogenicity after 2 passages in chickens.

Other species of chicken coccidia which have been cultivated in chick embryos include E. brunetti (Long, 1966; Shibalova, 1969, 1970), E. mitis (Shibalova, 1970), E. mivati (Long, 1966), E. necatrix (Shibalova, 1970), and E. praecox (Shibalova, 1970). All of these species were shown to complete their endogenous cycles within the epithelial cells of the chorioallantoic membrane.

Fitzgerald (1970) successfully demonstrated the development of E. stiedae, a mammalian coccidium of rabbits, in the chorioallantoic membrane of chick embryos. He inoculated 150,000 sporozoites into the allantoic cavity and opened the eggs each day beginning 2 days after inoculation. Schizonts and oocysts were not observed in the allantoic fluid or in the chorioallantoic membrane. Early

gametocytes, however, were observed, and were shown to be nearly identical to those found in the bile duct epithelium of infected rabbits.

In vitro cultivation of chicken and mammalian coccidia in cell cultures has been accomplished by numerous investigators (Doran, 1973). However, no work with the cultivation of E. dispersa either in embryos or in cell cultures has been reported at the present time.

MATERIALS AND METHODS

Preparation of oocyst inoculum. The original inoculum used in this study was obtained on July 10, 1972 from a single mature Bobwhite quail (Colinus virginianus) belonging to a quail colony housed at Drake University. Droppings from this infected bird were strained through a sieve and mixed thoroughly in a 2.5% aqueous solution of potassium dichromate. The mixture was then poured into a fingerbowl to a depth of approximately 5 mm and incubated for 5 days at room temperature (22° C) to facilitate sporulation of the oocysts. Sporulated oocysts from this suspension were then freed of potassium dichromate by repeated centrifugation (2,000 rpm) in tap water. The cleaned oocysts were resuspended in tap water at a concentration of 1,000 oocysts per ml. Two mls from this cleaned oocyst suspension were then

inoculated per os, using a syringe and feeding tube, into a noninfected Bobwhite. Droppings from this inoculated bird were collected at 12-hour intervals over a period of 12 days and the oocysts contained in these droppings were prepared according to the above mentioned method. In order to obtain an even larger quantity of inoculum, 15,000 to 30,000 of these sporulated oocysts were inoculated into each of 7 additional Bobwhites. From these 7 birds, fecal samples containing heavy concentrations of oocysts were collected, and the oocysts which they contained were prepared as mentioned above, except that the sporulated oocyst suspensions were pooled and sedimented in 1-liter beakers for 24 hours at room temperature (22° C). The supernatant was then siphoned off with a rubber tube, and the remaining oocysts were cleaned and concentrated by using the following Petri dish flotation technique: 5 ml portions of the concentrated oocyst suspension were placed in each of several inverted Petri dish lids filled 2/3 full with 50% Sheather's solution. Petri dish bottoms were floated on the surface of the suspension contained in the inverted lids for a period of 1 hour to allow the oocysts to rise in the Sheather's solution and to adhere to the underside of the floating Petri dish bottoms. The bottoms were then removed and the adhering oocysts washed off into a separate jar with the aid of a jet of water from a wash bottle. The bottoms were refloated on

the same suspensions, and after an additional $\frac{1}{2}$ hour, the washing process was repeated in order to collect any oocysts which may have remained in the Sheather's solution after the first washing. The cleaned oocyst suspension was then sedimented several times at 12-hour intervals in large quantities of distilled water in order to remove all traces of sugar. The oocysts were resuspended in a 2.5% aqueous potassium dichromate solution to form a stock suspension and stored in a refrigerator at 4° C. When used, the oocysts were 1 to 6 months old. Prior to use, oocysts from the stock suspension were cleaned and concentrated according to the methods of Fayer and Hammond (1967), i.e., 15 mls of oocyst suspension were layered over 20 mls of 33% Sheather's solution in a 50-ml centrifuge tube and centrifuged at 2,000 rpm for 5 minutes. Oocysts were removed at the water-sugar interface with a Pasteur pipette and washed free of sugar by repeated centrifugation (2,000 rpm) in distilled water. The oocysts were then disinfected in undiluted Clorox (5.25% sodium hypochlorite) overnight at room temperature (22° C) (Wagenbach et al., 1966). The Clorox was removed by repeated centrifugation (2,000 rpm) with sterile Tyrode's solution.

Preparation of sporozoite inoculum. Sporocysts were released from the oocysts suspended in Tyrode's solution by mechanically rupturing the oocyst walls with the aid of a

Dounce 7-ml tissue grinder. Homogenation for 2 to 4 minutes in sterile Tyrode's solution gave sufficient oocyst breakage. The sporocysts were excysted in a medium containing 5.0% fresh or frozen whole chicken bile and 0.25% trypsin (Fisher Scientific Co.) in sterile Tyrode's solution. Excellent excystation was achieved when 10 mls of sporocyst suspension were mixed with 5 mls of excystation medium. To ensure maximal excystation, this mixture was incubated for approximately $1\frac{1}{2}$ hours at 39.5° C. The excystation medium was removed by washing the sporozoites 3 times by centrifugation at 3,000 rpm in sterile Tyrode's solution.

Inoculation of embryos. Seventy-four embryonated Bob-white quail eggs, incubated at temperatures between 39° and 40° C were inoculated with sporozoites within 30 minutes after the sporozoites were washed with Tyrode's solution. Prior to inoculation, and after the eggs had incubated for 10 to 12 days, the viability of the embryos was determined by candling. Each egg was candled separately using a 35W Bausch and Lomb Nicholas illuminator. After candling, the egg was secured in a clamp on a ring stand so that the long axis of the egg was parallel to the table. The site for inoculation was selected from an area on the uppermost surface of the egg near the edge of the air sac. The site was disinfected with a 10% iodine-alcohol solution and punctured with the aid of a variable speed dental drill. A 0.15-ml

portion of the sporozoite suspension containing 1,000 to 44,000 sporozoites was injected into the allantoic cavity, using a tuberculin syringe equipped with a 25-gauge, $\frac{1}{8}$ -inch needle. For each 0.15-ml portion of sporozoite suspension, 6,000 iu of Penicillin and of Streptomycin were added in order to retard bacterial growth (Long, 1970). The number of sporozoites inoculated was determined with a hemocytometer. During inoculation, the needle was introduced parallel to the long axis of the egg for a distance of $\frac{1}{4}$ inch. After inoculation, the site of injection was sealed with collodion.

Of the 74 embryonated eggs which were inoculated, only 45 survived. Two viable eggs were opened at each 12-hour interval between $\frac{1}{2}$ and $2\frac{1}{2}$ days, and between $5\frac{1}{2}$ and 10 days, after inoculation. Between days 3 and 5, 3 eggs were opened at each 12-hour period following inoculation.

Preparation of chorioallantoic membranes and life cycle stages for study. The eggs were initially opened at the air sac with a sharp probe. Forceps were then used to widen the opening and to peel back the shell and shell membrane. With the aid of a Pasteur pipette, the allantoic fluid was removed and examined with a microscope at 100 X for the presence of non-sporulated oocysts and other life cycle stages. Oocysts recovered from the allantoic fluid were sporulated in a thin layer (5 mm) of 2.5% aqueous potassium dichromate solution for 5 days at room temperature

(22° C). These oocysts were cleaned as before and inoculated into a 3-month-old Bobwhite in order to demonstrate their infectivity. The chorioallantoic membranes from infected embryos were prepared for study as follows: Portions of the freshly removed chorioallantoic membrane were floated in 0.1 M phosphate-buffered saline solution onto standard microscope slides so that the cell layer lining the allantoic cavity was on top. Attachment of the chorioallantoic membrane to the slide was facilitated with the use of Meyer's albumin. The membranes were then fixed by placing the slides in Zenker's fluid for 30 minutes and rinsing them in tap water for 5 hours. The slides were then stained with hematoxylin and eosin (H and E).

Oocysts from infected Bobwhites were prepared for study by suspending about 1 gm of feces in Sheather's solution in a 15-ml centrifuge tube and centrifuging the suspension at 2,000 rpm for 5 minutes. Following centrifugation, the loop of a flame-sterilized inoculating needle was used to transfer oocysts from the surface of the Sheather's solution within the centrifuge tube to a microscope slide. A cover-slip was placed over the transferred solution, and the slide was examined for oocysts.

Living sporozoites, removed from flasks during the excystation procedure, and living merozoites, pipetted from the allantoic cavity, were placed on microscope slides,

covered with coverslips, examined, and measured.

Observations of living stages and stages which were prepared histologically were made with the aid of a compound microscope equipped with achromatic objectives and an ocular micrometer. Photographs were made with a Zeiss photomicroscope equipped with Neofluar objectives. A sporulated oocyst was drawn to scale using an average-sized oocyst as a model.

All measurements are expressed in micrometers, unless otherwise stated, with the ranges given in parentheses, when appropriate.

Experimental birds. Quail used in this study were separated into 2 groups with 1 group being used for oocyst production and the other for egg production. Both groups were maintained separately in wire-bottomed cages. Each bird used for the production of oocysts was reared separately and maintained ad libitum on commercial medicated game bird feed, water, and gravel. One week prior to inoculation a commercial non-medicated feed was substituted for the medicated one. The group used for egg production was comprised of 1 male and 5 females. A commercial medicated game bird feed containing 20% protein was fed ad libitum to this group throughout the study. The paper beneath the wire floor of the cages of those birds used for egg production was changed weekly, while the paper beneath the wire floor of the cages

of those used for oocyst production was changed daily. All quail were maintained at a constant temperature of 22° C, and the lighting was timed to create a daylight period of 14½ hours (7:00 a.m. to 9:30 p.m.). Under these controlled conditions, Bobwhites were able to produce eggs during the entire year. Freshly laid eggs were held in a container for periods up to 7 days at temperatures between 18° and 24° C in order to arrest the growth of the embryos so that the times at which they were to begin development prior to inoculation, could be synchronized.

Prepatent and patent periods. In order to determine the prepatent and patent periods, 8 Bobwhite quail, ranging in age from 1 week to 1 year, were inoculated with 1,000 to 30,000 oocysts each. The birds were maintained on non-medicated feed at least 1 week prior to inoculation, and were found to be free from infection during this time. Droppings from inoculated birds were collected at 8:00 a.m. and 4:00 p.m. daily, beginning 48 hours post-inoculation, and were examined for oocysts according to the methods mentioned earlier.

Cross-transmission studies. In order to determine the infectivity of E. dispersa for avian species other than the Bobwhite quail, two 2-month-old domestic turkeys (Meleagris gallopavo), two 2-week-old White Leghorn chickens (Gallus domesticus), and two 2-week-old Japanese quail (Coturnix

coturnix japonicum) were inoculated with sporulated oocysts of E. dispersa. These birds were held in wire-bottomed cages with each pair being caged separately and given non-medicated feed for 1 week prior to inoculation. At the end of the 1 week period, fecal samples were taken from each of the 3 cages and examined for coccidian oocysts. Having confirmed that the birds were negative for coccidia, they were inoculated per os with sporulated oocysts of E. dispersa as follows: The turkeys were each given approximately 36,000 oocysts, and their droppings were examined for oocysts each day for 14 days post-inoculation. The chickens were fed approximately 100,000 oocysts each, and their fecal samples were checked daily for 10 days after inoculation. The Japanese quail were given 30,000 oocysts each, with their droppings being examined for oocysts each day for 12 days post-inoculation.

DATA

Eimeria dispersa Tyzzer, 1929, Redescribed

Redescription of sporulated oocysts. Oocysts subspherical to ovoid (Figs. 1 and 2). Outer surface of oocyst wall smooth. Wall of intact oocyst about 0.8 (0.7 to 1.0) thick with brownish-yellow outer layer, reddish-brown inner layer. Micropyle absent. Single polar granule present in 80% of oocysts as flattened body, lying in micropylar position

against oocyst wall. Oocyst residuum absent. Seventy-five sporulated oocysts averaged 22.4 (18.9 to 27.5) by 18.5 (14.8 to 21.9); length/width ratio, 1.2 (1.1 to 1.3). Sporocysts assymmetrically ellipsoidal with one side appearing more convex than the other, and with Stieda body slightly displaced from longitudinal axis of the sporocyst. Substiedal body present. Sporocyst residuum present as membrane-bound body containing numerous spherical granules of uniform size. Seventy-five sporocysts averaged 14.1 (11.5 to 16.5) by 6.7 (5.0 to 8.0). Freshly excysted sporozoites (Fig. 3) slightly wider at posterior end than at anterior end, with 1 large refractile body averaging 7.7 (6.0 to 8.5) by 2.6 (2.5 to 3.0) present at posterior end, and 1 small refractile body averaging 4.1 (3.0 to 5.5) by 2.4 (2.0 to 2.5) present at anterior end. Nucleus usually visible about 3.8 from posterior end of sporozoite. Fifty extended sporozoites averaged 13.8 (12.0 to 16.0) by 2.7 (2.5 to 3.0); 50 flexed sporozoites averaged 11.5 (10.0 to 13.5) by 2.6 (2.5 to 3.0).

Development of Endogenous Stages

Development of all endogenous stages occurred within the chorioallantoic membrane of Bobwhite quail embryos. Two kinds of schizonts, 1 large and 1 small, and each probably belonging to a different generation, were observed. The

large schizonts were seen beginning with the second day post-infection and the small schizonts beginning with the fourth day post-infection. Gametogony was observed as early as 4 days post-inoculation and oocysts were found after 8 days. The endogenous stages were found to lie predominantly along blood vessels within the chorioallantoic membrane although some were observed to lie within the tissues between the blood vessels, and some stages were observed free within the fluid of the allantois.

Extracellular and intracellular sporozoites. Freshly excysted sporozoites (Fig. 3) were motile, showing typical gliding, flexing, probing, and pivoting movements.

Fresh chorioallantoic membranes from 4 embryos were studied $\frac{1}{2}$, 1, and $1\frac{1}{2}$ days after inoculation, but neither penetration of cells by sporozoites nor intracellular sporozoites were observed (Table 1). However, numerous free sporozoites were recovered from the allantoic fluid during this time.

In fixed preparations, intracellular sporozoites were first observed at 2 days post-inoculation lying in the cytoplasm of epithelial cells lining the allantoic cavity. The sporozoites were located close to the host cell nucleus and lay within a parasitophorous vacuole. Most parasitized cells contained 1 sporozoite, but some contained 2 to 5 sporozoites. Intracellular sporozoites were present in

greatest concentration on days 2 and $2\frac{1}{2}$ and gradually decreased in prevalence until day $9\frac{1}{2}$ when only 1 sporozoite was observed (Table 1).

Intracellular sporozoites were observed to lie either in a slightly curved position (Fig. 4) within the host cell cytoplasm or in a characteristic "U-shaped" position (Fig. 5). Sporozoites within cells appeared consistently shorter and wider than free sporozoites. Fifty intracellular sporozoites on day 2 averaged 7.8 (7.0 to 8.5) by 4.0 (3.5 to 4.2). Most intracellular sporozoites had a large posterior refractile body and a smaller anterior refractile body. Fifty posterior refractile bodies from 2-day infections were 3.7 (3.5 to 4.0) by 2.0 and 50 anterior refractile bodies were 2.4 (2.0 to 2.5) by 2.0. The nucleus of the sporozoite, when seen, was centrally located between the anterior and posterior refractile bodies. Both the anterior and posterior refractile bodies stained highly eosinophilic with H and E while the nucleus appeared intensely basophilic; the cytoplasm appeared slightly eosinophilic. In infected cells observed 2 days post-inoculation, the chromatin of the host cell nucleus appeared more intensely stained than normal (Fig. 4). The nucleolus of the host cell was also more readily discernible at this time in infected cells.

Sporozoite-shaped schizonts. Two to $9\frac{1}{2}$ days after inoculation, sporozoite-shaped schizonts (Fig. 6) were

observed. Their numbers occurred with greatest frequency on day $2\frac{1}{2}$ and gradually decreased until day $9\frac{1}{2}$ after which they were no longer seen. Twenty-five sporozoite-shaped schizonts, from 2- to $9\frac{1}{2}$ -day infections, averaged 10.0 (9.0 to 11.0) by 3.7 (3.0 to 4.0). The number of nuclei present in 25 sporozoite-shaped schizonts ranged from 2 to 10. These nuclei stained intensely basophilic with H and E. In most sporozoite-shaped schizonts, both posterior and anterior refractile bodies were observed. The posterior refractile bodies appeared subspherical to spherical in shape, and stained eosinophilic with H and E. Twenty-five posterior refractile bodies averaged 2.6 (2.2 to 2.8) by 2.4 (2.0 to 2.5). Anterior refractile bodies were usually inconspicuous and stained lightly eosinophilic; they averaged about 1.5 in diameter. Crescent bodies were not seen in any of the developmental stages.

Sporozoite-shaped schizonts were always observed singly within the cytoplasm of the host cell. No apparent alterations in host cell structure were observed in association with these stages.

Immature schizonts. Sporozoite-shaped schizonts appeared to grow shorter and more rounded as the number of nuclei increased. Immature schizonts of this shorter and more rounded shape (Fig. 7) were first seen 2 days after inoculation (Table 1). These shorter, more rounded schizonts

increased in prevalence until day $3\frac{1}{2}$ when they were extremely numerous and decreased in numbers until day $9\frac{1}{2}$ when only a few were seen. Twenty-five immature schizonts of this type averaged 9.9 (7.4 to 12.7) by 7.7 (4.9 to 10.8). Eighty-five percent of all immature schizonts had 1 relatively large subspherical to spherical refractile body. Refractile bodies from 25 immature schizonts averaged 2.5 (2.0 to 3.0) by 2.0 (1.5 to 2.5). Fifteen percent of the immature schizonts observed had a second, smaller, spherical refractile body averaging about 1.0 in diameter. Both kinds of refractile bodies stained intensely eosinophilic with H and E, and the cytoplasm of the schizont appeared lightly eosinophilic. The nuclei of these immature schizonts ranged from 10 to 24 in number, and stained highly basophilic. Two and one-half days after inoculation, immature schizonts containing refractile bodies became less frequent, and the schizonts began to form merozoites.

Development of immature schizonts occurred within the cytoplasm of the host epithelial cells. A very conspicuous parasitophorous vacuole surrounded the parasite during this stage; in many cases, the infected host cell appeared larger than normal in size and the host cell nucleus appeared smaller than normal.

Large schizonts. Mature schizonts of the large type appeared $2\frac{1}{2}$ days post-inoculation and persisted through day

10 (Table 1). These schizonts were larger in size and contained more merozoites than the smaller type, which were first observed on day 4, and probably belonged to a different generation than the smaller type. All large schizonts studied were found in the cytoplasm of the epithelial cells of the chorioallantoic membrane. With the formation of merozoites, large schizonts occupied much of the host cell cytoplasm and often obscured the host cell nucleus which appeared smaller than normal. The host cells also appeared greatly enlarged and blebs, or vacuolated areas, were sometimes visible within the cytoplasm near the surface of the infected cells (Fig. 8). The parasitophorous vacuole was less conspicuous in mature large schizonts. Large schizonts (Fig. 8) were seen in greatest numbers on days $3\frac{1}{2}$, 4, and $4\frac{1}{2}$, with the smallest concentrations of these schizonts occurring on days $9\frac{1}{2}$ and 10.

Seventy-five large schizonts averaged 16.7 (10.0 to 22.7) by 12.4 (6.5 to 18.0). In schizonts containing radially arranged merozoites, an average of 24 (13 to 30) merozoites were observed radiating from a centrally or eccentrically located residual body. Twenty-five residual bodies averaged 5.2 (4.2 to 7.4) in diameter. In mature, large schizonts containing randomly arranged merozoites, 75 merozoites averaged 6.1 (4.3 to 7.7) by 1.2 (0.8 to 1.6). With H and E, the cytoplasm of developing and mature merozoites

stained lightly eosinophilic and the residual body of large schizonts appeared basophilic.

Free merozoites from large schizonts. Merozoites, probably belonging to schizonts of the large type, were found repeatedly in the extracellular spaces of the chorio-allantoic membrane $3\frac{1}{2}$ to $9\frac{1}{2}$ days post-inoculation. At no time were merozoites from large schizonts found free in the allantoic fluid. In situ, these merozoites appeared short and either slightly curved or fully extended (Fig. 9). Twenty-five fixed specimens averaged 6.9 (6.5 to 7.0) by 1.5 (1.0 to 1.5). The cytoplasm of these merozoites stained lightly eosinophilic with H and E, with their nucleus staining intensely basophilic.

Small schizonts. Small schizonts, probably belonging to a different generation than the large schizonts, appeared 4 days after inoculation and were observed through day 10 (Table 1). These schizonts were most prevalent on day $4\frac{1}{2}$, but were also common on days 4, 5, and 8. Only a few small schizonts could be found on days 9, $9\frac{1}{2}$, and 10. These schizonts (Fig. 10) developed in parasitophorous vacuoles in the cytoplasm of epithelial cells of the chorioallantoic membrane. The parasitophorous vacuole appeared distinctly visible throughout the development of the schizont. Infected host cells did not appear to be greatly enlarged, and no blebbing or vacuole formation at the margin of the host cell

was observed. The developing schizont was usually pressed against the host cell nucleus (Fig. 10). The nucleolus of infected cells appeared more basophilic with H and E than non-infected cells which had lighter staining nucleoli.

Twenty-five small schizonts averaged 9.9 (7.0 to 13.5) by 7.9 (5.5 to 10.0). A single eccentric or central residual body was present and stained moderately basophilic with H and E. Twenty-five residual bodies averaged 1.7 (1.5 to 2.0) in diameter. In mature schizonts, merozoites usually lay parallel to each other within the schizont. In 50 of these small schizonts, the average number of merozoites per schizont was 12 (8 to 17). In mature small schizonts containing randomly arranged merozoites, 50 merozoites averaged 4.6 (3.5 to 5.5) by 1.0 (0.9 to 1.2). The cytoplasm of all merozoites within small schizonts stained moderately eosinophilic with H and E and the residual body stained intensely basophilic.

Free merozoites from small schizonts. Large numbers of merozoites, probably from small schizonts, were found free in the extracellular spaces (Fig. 11) and in the allantoic fluid (Fig. 12) at 4 to 10 days post-infection. Both fixed and living merozoites of this type appeared long, slender, and slightly curved. Twenty-five living merozoites from the allantoic fluid measured 8.5 (8.0 to 9.5) by 1.5 (1.3 to 1.8). The cytoplasm of specimens fixed in situ within the chorioallantoic membrane, stained moderately eosinophilic with H and E.

Microgametocytes. Sexual stages were also observed in epithelial cells of the chorioallantoic membrane. Microgametocytes appeared most frequently at 8 days post-inoculation, and were observed to occur until the end of the experiment at 10 days (Table 1). One mature microgametocyte, however, was seen on day 4.

In developing microgametocytes (Figs. 14, 15, 16), the nuclei appeared to be randomly distributed, with the nuclei decreasing in size as the number of nuclei increased. The nuclei of young microgametocytes stained intensely basophilic with H and E and the cytoplasm was moderately eosinophilic. Mature microgametocytes contained randomly arranged microgametes which were too numerous to count. As the microgametocytes matured, the parasitophorous vacuole appeared to become consistently larger. Compared to noninfected cells, infected cells were extremely enlarged, and in nearly mature microgametocytes, the peripheral portion of the host cell cytoplasm appeared agranular (Fig. 15). Twenty-five mature microgametocytes measured 18.7 (16.2 to 21.0) by 14.9 (10.0 to 17.5).

Macrogametes. Macrogametes were observed to undergo development in epithelial cells of the chorioallantoic membrane. Macrogametes were present between days 8 and 10 post-inoculation (Table 1). The nucleus of immature macrogametes stained basophilic with H and E, and the cytoplasm of the

parasite stained moderately eosinophilic; the cytoplasm appeared highly granular (Fig. 17). No satellite body was present in any of the macrogametes observed.

Mature macrogametes (Fig. 18) contained numerous large eosinophilic granules at the periphery as well as smaller basophilic granules throughout the cytoplasm. Macrogametes were surrounded by a parasitophorous vacuole which became larger as the macrogamete matured. Infected cells were greatly enlarged and the periphery of the host cell cytoplasm appeared agranular as with host cells containing microgametocytes. Twenty-five mature macrogametes were 19.2 (17.5 to 20.5) by 15.0 (14.0 to 16.0).

Embryo-derived oocysts. Oocysts formed within epithelial cells of the chorioallantoic membrane were observed at 8, 9, and 9½ days post-inoculation (Table 1). Twenty of these oocysts, in situ, measured 23.3 (21.5 to 25.0) by 18.6 (17.5 to 20.0). Of those oocysts measured, all lay within their host cells, and were bounded by a parasitophorous vacuole (Fig. 19). The wall of the oocyst appeared slightly basophilic with H and E.

Non-sporulated oocysts (Fig. 20) were found free in the allantoic fluid at 9 and 9½ days after inoculation. Four infected eggs, from each of which approximately 3 ml of allantoic fluid was extracted, were positive for non-sporulated oocysts. Three of the 4 eggs contained 7,000 oocysts per ml

of allantoic fluid and 1, 4,000 per ml. Fifty of these non-sporulated oocysts averaged 19.8 (17.5 to 21.0) by 17.0 (15.0 to 20.0).

A comparison between oocysts obtained from adult quail and those obtained from embryos, showed the latter to be smaller than those obtained from adults. The walls of embryo-derived oocysts appeared approximately $\frac{1}{2}$ as thick as those obtained from adults, and were much less refractile.

Host tissue response. The chorioallantoic membranes, upon removal from the eggs, showed no macroscopic lesions, even in heavily infected eggs. Microscopically, slight hyperemia and hemorrhaging was observed in scattered areas near large blood vessels. This was noticed on those days in which mature schizonts and/or gametocytes were present.

Host cells began to show alterations with the formation of merozoites in large and small schizonts, and when gametocytes approached maturity. These alterations included increased size of the host cell nucleus and increased vacuolization of the host cell cytoplasm surrounding the parasite.

Throughout the study, beginning 2 days post-inoculation, numerous heterophiles infiltrated the parasitized regions of the chorioallantoic membrane. Also, many of the large schizonts became surrounded with fibrocytic cells which appeared to wall-off the schizonts from the surrounding tissue (Fig. 13). These encapsulated schizonts were observed as

early as $4\frac{1}{2}$ days post-inoculation and throughout the remainder of the study. Encapsulated schizonts were most prevalent on day 8. Small schizonts did not appear to become encapsulated.

Prepatent and patent periods. In those birds used for inoculum, oocysts were first observed in the feces between 4 and $4\frac{1}{2}$ days after inoculation regardless of the number of oocysts inoculated. The only clinical symptom observed in these birds was slight diarrhea which occurred approximately 24 hours prior to oocyst passage, and which persisted through the first day of the patent period. The number of oocysts passed during the patent period increased until day 6, remained at a high level from day 6 through day 10, then decreased gradually until day 15 when only a few oocysts were present in fecal samples. The collection of fecal samples was terminated after day 15.

When 7,000 embryo-derived oocysts were given per os to a 3-month-old Bobwhite, the prepatent period was delayed until 6 days after inoculation. No clinical symptoms were observed in this bird during the prepatent or patent periods. This suggests that passage of E. dispersa through an embryo may delay the period of infectivity or the life cycle of the parasite.

Host specificity. Of the 2 turkeys, 2 chickens, and 2 Japanese quail inoculated with E. dispersa from adult

Table 1. Endogenous stages of *Eimeria dispersa* present in extraembryonic tissues of Bobwhite quail embryos at various times after sporozoite inoculation.

DEVELOPMENTAL STAGE	Days after inoculation																			
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
Intracellular sporozoites	—	—	—	+	+	O	+	+	+	+	O	O	O	O	O	+	O	+	+	—
Sporozoite-shaped schizonts	—	—	—	+	+	O	+	+	+	+	O	O	O	O	O	+	O	+	+	—
Immature large schizonts	—	—	—	+	+	O	+	+	+	+	O	O	O	O	O	+	O	+	+	—
Mature large schizonts	—	—	—	—	+	O	+	+	+	+	O	O	O	O	O	+	O	+	+	+
Mature small schizonts	—	—	—	—	—	O	—	+	+	+	O	O	O	O	O	+	O	+	+	+
Microgametocytes	—	—	—	—	—	O	—	+	—	—	O	O	O	O	O	+	O	+	+	+
Macrogametes	—	—	—	—	—	O	—	—	—	—	O	O	O	O	O	+	O	+	+	+
Oocysts ¹	—	—	—	—	—	O	—	—	—	—	O	O	O	O	O	+	O	+	+	—

¹ Present either in the chorioallantoic membrane or allantoic cavity or both.

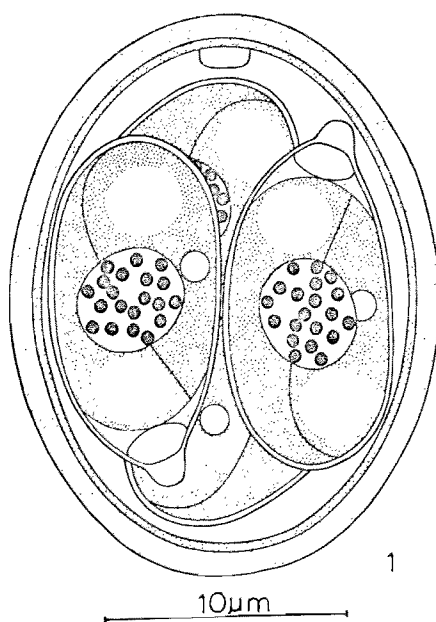
+ = Stage present.

— = Stage absent.

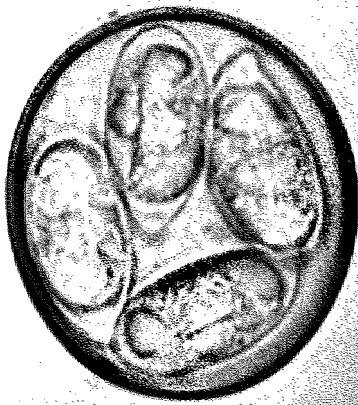
O = Those days inoculated but no stages found.

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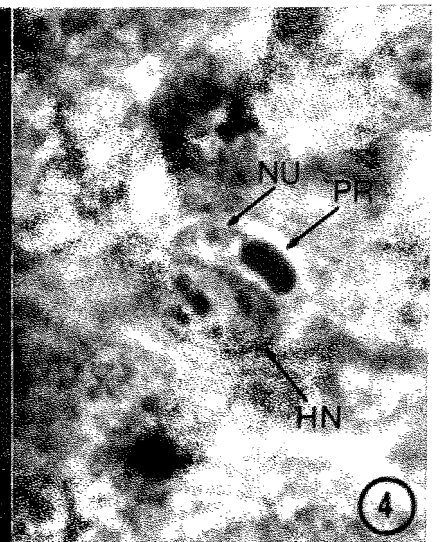
Figure 1. Sporulated oocyst of Eimeria dispersa.



Figures 2-10. Photomicrographs of developmental stages of Eimeria dispersa in the chorioallantoic membrane of Bobwhite quail embryos, fixed with Zenker's fluid and stained with H and E unless otherwise indicated. All photographs phase contrast unless otherwise indicated. Abbreviations: AR, anterior refractile body; HN, host cell nucleus; M, merozoite; NU, nucleus of parasite; Pk, posterior refractile body; PV, parasitophorous vacuole; RB, residual body. Days following description indicate number of days post-inoculation. 2. Sporulated oocyst of Eimeria dispersa from adult Bobwhite quail. Unfixed, bright field. X 2,200. 3. Unfixed sporozoite in excystation fluid. X 2,000. 4. Intracellular sporozoite, 2 days. X 2,400. 5. Intracellular sporozoite. Note "U-shaped" appearance, 2 days. X 1,400. 6. Sporozoite-shaped schizont. Note 1 of several nuclei, 2 days. X 3,000. 7. Immature large schizont at more advanced stage than sporozoite-shaped schizont. Note 1 of several nuclei, 2½ days. X 2,500. 8. Mature large schizont. Note central residual body and radial arrangement of merozoites. Also note refractile bodies (arrows), 2½ days. X 2,500. 9. Merozoites (arrows), probably from large schizonts, 4 days. X 2,000. 10. Mature small schizont. Note eccentric residual body and parallel arrangement of merozoites. Bright field, 4 days. X 2,400.



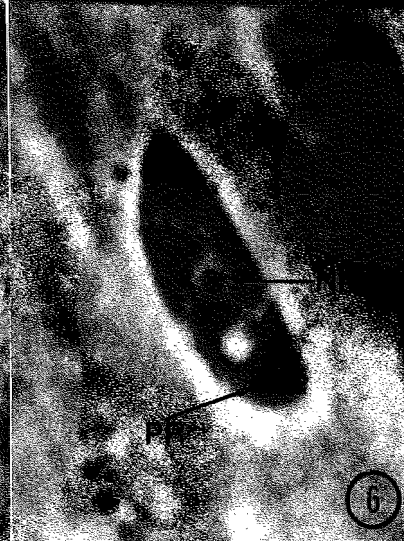
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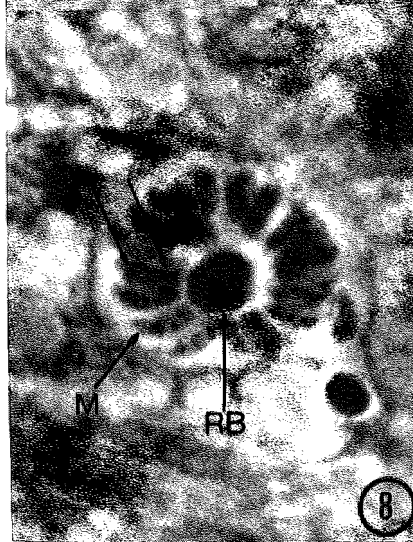
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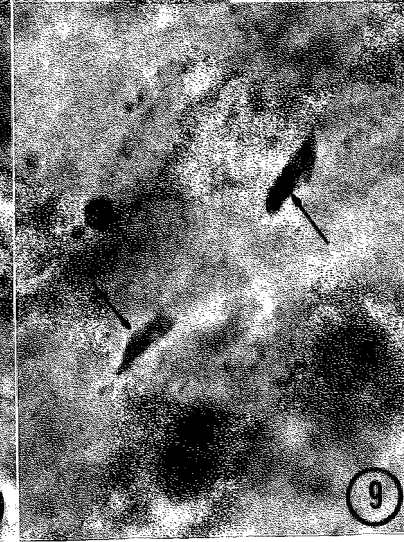
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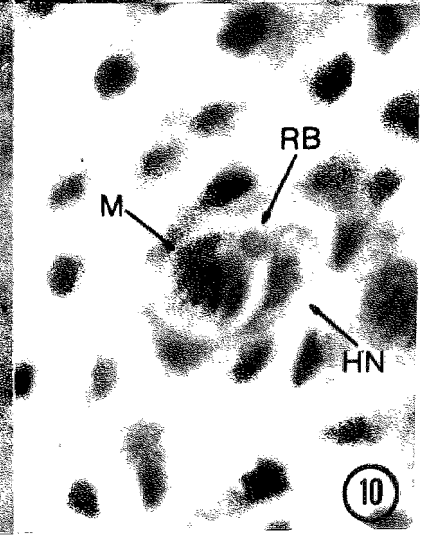
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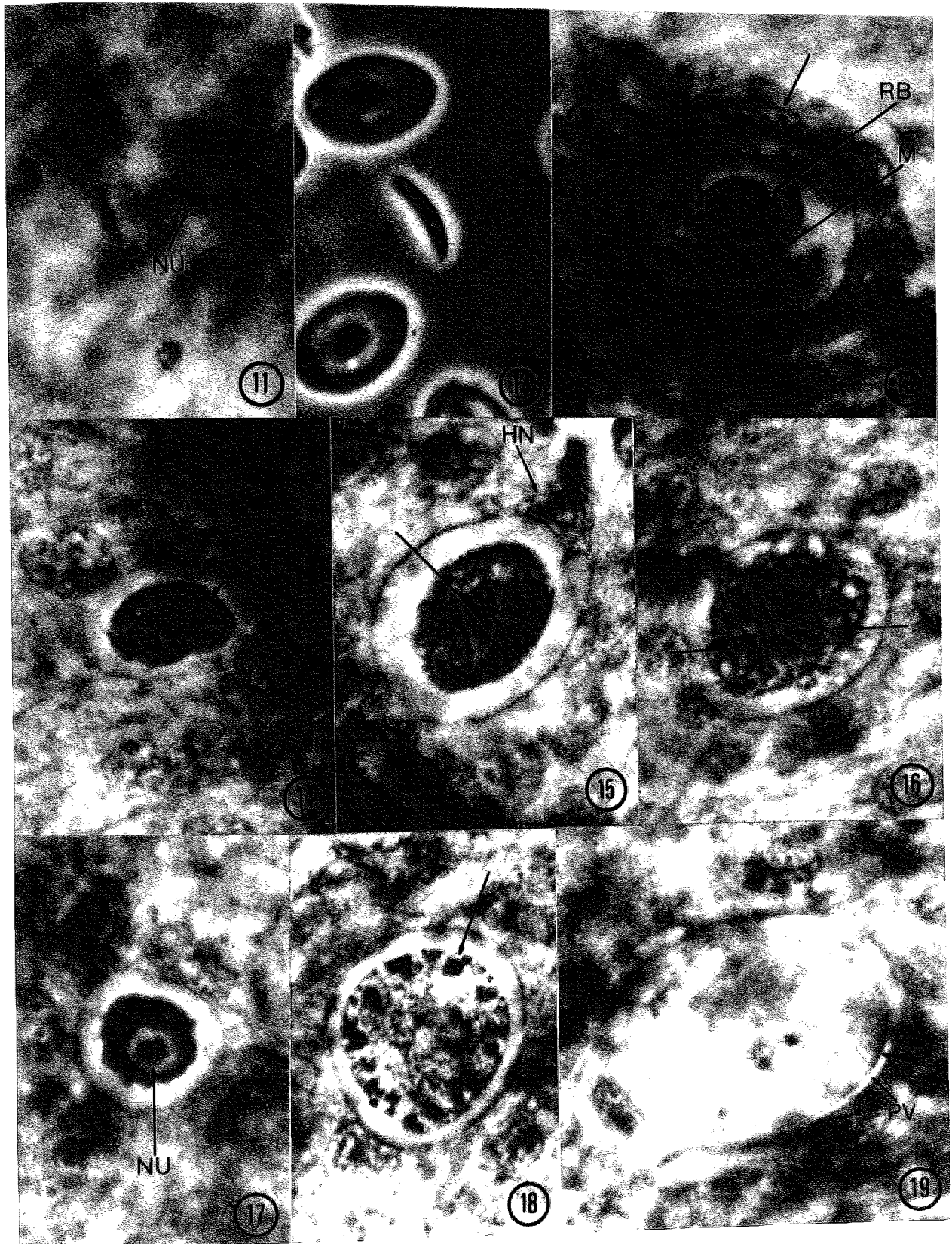


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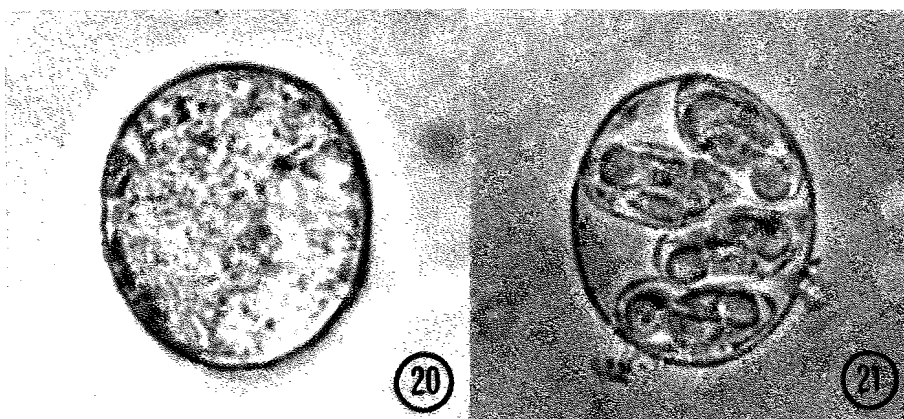


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Figures 11-19. Photomicrographs of developmental stages of Eimeria dispersa in the chorioallantoic membrane of Bobwhite quail embryos, fixed with Zenker's fluid and stained with H and E unless otherwise indicated. All photographs phase contrast unless otherwise indicated. Abbreviations: AR, anterior refractile body; HN, host cell nucleus; M, merozoite; NU, nucleus of parasite; PR, posterior refractile body; PV, parasitophorous vacuole; RB, residual body. Days following description indicate number of days post-inoculation. 11. Merozoites, probably from small schizonts, 8 days. X 2,200. 12. Unfixed merozoite, probably from a small schizont, found free in allantoic cavity, 9½ days. X 2,000. 13. Walled-off large schizont. Note envelope of cells (arrow) surrounding the degenerating schizont, 8 days. X 1,900. 14. Immature microgametocyte containing several large nuclei (arrow), 8 days. X 1,800. 15. Immature microgametocyte at more advanced stage than that shown in Fig. 14. Note large number of nuclei (arrow) of smaller size, 8 days. X 2,000. 16. Mature microgametocyte. Note darker stained microgametes (arrows), 8 days. X 2,000. 17. Immature macrogamete, 8 days. X 1,800. 18. Mature macrogamete showing numerous granules (arrow), 8 days. X 2,000. 19. Oocyst in situ. Note oocyst wall (arrow), 8 days. X 2,000.



Figures 20-21. Oocysts isolated from allantoic cavity of 20-day embryo inoculated 10 days earlier with 22,000 sporozoites of E. dispersa. Unfixed, bright field. 20. Non-sporulated oocyst. X 2,000. 21. Oocyst as in Fig. 20, after sporulation. X 2,000.



Bobwhites in the present study, none were observed to pass oocysts of E. dispersa in their feces.

DISCUSSION

Eimeria dispersa Tyzzer, 1929, and the eimerian parasite considered in the present study are believed to be the same species as evidenced by the following comparison of their biological characteristics. Tyzzer (1929) stated that the sporulated oocysts of E. dispersa were ovoid in shape and lacked a "well defined" polar granule. Oocysts of the present study were subspherical to ovoid in shape, and were found to contain a single "poorly defined" polar granule, i.e., the polar granule was relatively inconspicuous and was present in only 80% of the oocysts observed. According to Tyzzer, the sporulated oocysts of E. dispersa averaged 22.8 (17.2 to 26.4) by 18.8 (13.2 to 19.8); those of the present study measured 22.4 (18.9 to 27.5) by 18.5 (14.8 to 21.9). Tyzzer stated that the sporocysts of E. dispersa were large and sometimes asymmetrical, filling almost the entire oocyst. In the present study, sporocysts of E. dispersa were similarly large and asymmetrical, occupying nearly the whole oocyst. The prepatent period for E. dispersa, determined by Tyzzer, was 4 days. In the present study, the prepatent period ranged from 4 to $4\frac{1}{2}$ days. In histological sections, Tyzzer noticed that sporozoites in epithelial cells were not

immediately transformed into rounded trophozoites, but rather persisted as elongate forms until these forms reached a considerably larger size. These forms probably represented sporozoite-shaped schizonts. Sporozoite-shaped schizonts have been reported in both mammalian and avian coccidia (Doran and Vetterling, 1968; Chobotar et al., 1969; Clark and Hammond, 1969; Speer et al., 1970). Stages resembling sporozoite-shaped schizonts were also seen in the present study and are believed to be the "elongate forms" described by Tyzzer. Tyzzer also described 2 generations of schizonts; schizonts belonging to the first generation ranged from 6.0 to 24.0 in diameter, and those of the second generation ranged between 11.0 and 13.0 in diameter. In the present study, large schizonts averaging 16.7 (10.0 to 22.7) by 12.4 (6.5 to 18.0) were observed, while smaller schizonts averaging 9.9 (7.0 to 13.5) by 7.9 (5.5 to 10.0) were also present; these probably correspond to the first- and second-generation schizonts described by Tyzzer. Tyzzer (1929) found that mature quail heavily infected with E. dispersa were usually in good condition and of normal weight, while heavily infected young 1-week-old quail usually died. Similarly, in the present study, heavily infected mature Bobwhites remained almost normal, except for slight diarrhea just prior to passing oocysts, while heavily infected juvenile quail in the first

or second week of life succumbed to the infection. These comparative data strongly support the present redescription of E. dispersa.

In addition to E. dispersa, other species of coccidia have been reported from the Bobwhite quail. Waggoner (1967) reported an E. species from Bobwhites which had oocysts similar in size and shape (broadly ovoid; 20.0 (15.4 to 26.9) by 17.9 (14.1 to 22.4)) to those found in the present study. However, the E. species reported by Waggoner differs from E. dispersa as follows: Micropyle present in freshly passed oocysts. Sporocysts ovoid, averaging 9.0 (5.3 to 10.5) by 4.6 (4.0 to 5.3). Sporozoites slightly curved, averaging 9.0 (6.7 to 10.6) by 4.6 (4.0 to 5.3). First-generation schizonts 10.6 (5.8 to 16.0) in diameter, containing from 10 to 25 merozoites; second-generation schizonts 14.6 (10.6 to 16.0) in diameter, containing approximately 30 to 40 merozoites. Free merozoites of 2 sizes, with larger, free merozoites measuring approximately 11.7 by 1.6 to 4.0 and smaller, free merozoites measuring approximately 6.0 by 1.6. Microgametocytes averaged 16.4 (10.6 to 23.9) and macrogametes were not described. Prepatent period about 3 to 3½ days.

Waggoner (1967) also mentioned a second E. species from Bobwhite quail which was reported to him through a personal communication with Battle. The oocysts of this second

species reported by Waggoner (1967) were similar in size and shape (broadly ovoid; 21.9 (14.1 to 25.6) by 17.4 (11.5 to 20.5)) to those of E. dispersa, but differed in other features as follows: Sporozoites approximately 7.6 by 4.2. Schizonts approximately 11.0 in diameter with merozoites ranging in size from 5.3 to 10.6 by 1.8 to 4.0. Prepatent period about $3\frac{1}{2}$ days.

Two other species of coccidia, E. tenella (Venard, 1933) and E. acervulina (Venard, 1933), have been reported from the Bobwhite. According to Hawkins (1952), Levine (1953), and Pellérdy (1965), however, the only properly described species occurring in the Bobwhite is E. dispersa.

At the present time, cross-transmission studies concerning E. dispersa appear to be in a state of confusion. A comparison of the results of cross-transmission studies conducted with E. dispersa by Tyzzer (1929) and those conducted with E. dispersa in the present study, shows certain dissimilarities. Tyzzer (1929) claimed to have infected domestic turkeys and Rhode Island Red chickens with E. dispersa from Bobwhite quail. Of the 6 turkeys he inoculated, 3 passed oocysts identical to those which were inoculated, and 3 passed oocysts different from those inoculated. Of the 12 chickens inoculated, 6 became infected, but most of the oocysts were morphologically dissimilar to those of the original inoculum; Tyzzer attributed these variable results

to strain differences in the coccidia used. However, cross-transmission studies are difficult to control, even under the most ideal conditions, and therefore, it seems more likely that the birds which Tyzzer inoculated, accidentally became contaminated with other species or were not given a pure inoculum. In the present study, domestic turkeys, White Leghorn chickens, and Japanese quail failed to become infected with E. dispersa when fed sporulated oocysts from adult Bobwhite quail. It is possible that both of the 2-month-old turkeys used in the present study had been naturally infected with E. dispersa, and therefore were immune at the time of inoculation. However, oocysts were not found in the feces of the 2 turkeys, 5 days prior to inoculation. Hawkins (1952) claimed to have obtained fecal material containing oocysts of E. dispersa from turkeys that were naturally infected with this species, and reportedly infected turkeys, Hungarian partridges, and Bobwhite quail with these oocysts. The prepatent periods observed in all 3 hosts lasted from 5 to 6 days. He stated that the increased length of the prepatent period beyond the 4-day period normally attributed to E. dispersa was probably due to the introduction of E. dispersa into an abnormal host. Hawkins was unable to infect pheasants or chickens with E. dispersa from the turkey. Hawkins (1952) failed to report whether the original fecal material he used contained only oocysts

of E. dispersa. It seems quite unlikely that such a pure inoculum could be obtained from an abnormal host in the field.

On the basis of the work done by Tyzzer (1929), by Hawkins (1952), and in the present study, it is felt that E. dispersa may be host specific for the Bobwhite quail, and that the oocysts which Hawkins (1952) obtained from turkeys probably belonged to a species other than E. dispersa.

In the present study, a delay in the prepatent period of E. dispersa cultivated in Bobwhite quail embryos was observed. In adult birds, the prepatent period of E. dispersa was 4 to $4\frac{1}{2}$ days, while in embryos, oocysts were first found at 8 days post-infection. The prepatent period in embryos may have been somewhat shorter since no infected eggs were available between days $5\frac{1}{2}$ and $7\frac{1}{2}$. The cycle, therefore, would have been delayed at least 1 day since no oocysts were observed through day 5. A similar delay of 2 and 3 days respectively were observed when chick embryos were inoculated with sporozoites of E. mivati and E. brunetti (Long, 1966).

When embryo-derived oocysts of E. dispersa were sporulated and given to a 3-month-old Bobwhite, oocysts appeared in the feces 6 days after inoculation. This is $1\frac{1}{2}$ to 2 days longer than the usual prepatent period for this species. This extended prepatent period was not observed in adult birds inoculated with embryo-derived sporulated

oocysts of E. tenella, E. brunetti, and E. mivati by Long (1966, 1973). It is not known why the prepatent period was prolonged in adult quail inoculated with oocysts from a single passage in embryos. This phenomenon appears to be peculiar only to E. dispersa.

In the present study, free merozoites were seen in the allantoic cavity, and endogenous stages were seen developing within cells lining the allantoic cavity. These observations are similar to those made by Long (1966) for E. tenella, E. brunetti, and E. mivati.

Long (1970) found a direct relationship between size of inoculum and mortality due to hemorrhage in embryos inoculated with sporozoites of E. tenella. In the present study, mortality in embryos probably resulted from bacterial contamination rather than from infection with the parasite, as there appeared to be no correlation between the amount of hemorrhage and the quantity of sporozoites inoculated.

Long (1973) discovered that embryo-passaged oocysts of E. tenella were smaller in size to those from adult chickens. This phenomenon was also observed in the present study. Freshly sporulated oocysts of E. dispersa from adult Bobwhites averaged 22.4 (18.9 to 27.5) by 18.5 (14.8 to 21.9), while those from embryos measured 19.8 (17.5 to 21.0) by 17.0 (15.0 to 20.0). Also, the walls of oocysts from embryos were approximately $\frac{1}{2}$ as thick as those from adults.

As stated earlier, the sporozoite-shaped schizonts of E. dispersa, found in the chorioallantoic membrane in the present study, are probably the same as the "elongate" stages described by Tyzzer (1929). Sporozoite-shaped schizonts have been reported for E. alabamensis (Sampson et al., 1971), E. auburnensis (Clark and Hammond, 1969), E. bilamellata and E. callospermophili (Speer et al., 1970), E. larimerensis (Speer and Hammond, 1970), E. magna (Speer and Hammond, 1971), and E. meleagrimitis (Doran and Vetterling, 1968). The sporozoite-shaped schizonts described in the present study most closely resembled those reported by Speer et al. (1970) for E. bilamellata and E. callospermophili. Sporozoite-shaped schizonts of E. bilamellata and E. callospermophili contained from 2 to 12 nuclei each; sporozoite-shaped schizonts of E. dispersa in the present study contained from 2 to 10 nuclei. E. bilamellata and E. callospermophili sporozoite-shaped schizonts rarely had an anterior refractile body, whereas E. dispersa usually contained a single, small, anterior refractile body. As the number of nuclei in E. bilamellata and E. callospermophili sporozoite-shaped schizonts increased, their size gradually decreased. This phenomenon was also observed in sporozoite-shaped schizonts in the present study. In most sporozoite-shaped schizonts of E. callospermophili, the posterior refractile body was considerably shorter and slightly wider

than in sporozoites of the same species. This was also found to be the case in the present study.

Sporozoites of E. dispersa were observed free in the allantoic fluid of embryos throughout the present study until day 9½ when only 1 free sporozoite was observed. Intracellular sporozoites, as noted in Table 1, appeared 2 days after inoculation, and were found until day 9½. No previous investigators appear to have reported the occurrence of extracellular or intracellular sporozoites with any Eimeria species cultivated in embryos. Many investigators, however, have reported survival times for sporozoites in tissue culture. Fayer and Hammond (1967) observed free, motile sporozoites of E. bovis for 18 days in cultures of bovine spleen and kidney cells. They also found intracellular sporozoites as late as 17 days after inoculation in bovine spleen and thymus cells, and as late as 18, 14, and 12 days respectively in cultured bovine kidney, intestinal, and testicle cells. Hammond and Fayer (1968) observed extracellular, motile E. bovis sporozoites daily for 6 days after inoculation in cultured mouse fibroblasts (L cells). Speer et al. (1970) discovered free, motile E. callospermophili sporozoites for a maximum of 48 hours in bovine cell cultures while extracellular, motile E. bilamellata sporozoites were seen in cultures as late as 8 days after inoculation. These investigators stated that E. callospermophili

sporozoites probably developed beyond the intracellular sporozoite stage during this relatively short period of time. Fayer and Hammond (1967) suggested that the temporary invasion of cells by sporozoites may contribute to their longevity in cell cultures. The evidence supporting this hypothesis is based on the observation that motile E. bovis sporozoites could be seen for a maximum of only 21 hours in media without cells (Fayer and Hammond, 1967), that E. bovis sporozoites were able to enter and leave cultured bovine cells (Fayer and Hammond, 1967), and that E. bovis sporozoites left Madin-Darby kidney (MDBK) cells 5 and 8 days after inoculation (Hammond and Fayer, 1968). Similarly, E. crandalis sporozoites have been reported to leave and enter cultured bovine cells for 8 days post-inoculation (DeVos et al., 1972). Since, in the present study, pieces of chorioallantoic membranes were placed in a fixative prior to examination, it was impossible to determine whether or not E. dispersa sporozoites entered and left epithelial cells, as observed for those species mentioned above. It would seem logical, however, that the sporozoites observed in the present study were intracellular part of the time since both extracellular and intracellular sporozoites were seen over a period of $9\frac{1}{2}$ days.

Speer and Hammond (1969) reported flexing in sporozoite-shaped schizonts of E. callospermophili in monolayer cultures.

When freed from the monolayer, sporozoite-shaped schizonts of E. callospermophili (Speer and Hammond, 1969) and E. larimerensis (Speer and Hammond, 1970) underwent gliding and flexing movements similar to that seen for sporozoites. Sporozoite-shaped schizonts of E. callospermophili (Speer and Hammond, 1969; Speer et al., 1970) have even been observed to penetrate new cells in a manner similar to that reported for sporozoites (Speer et al., 1970). That E. dispersa sporozoite-shaped schizonts were seen over a period of $7\frac{1}{2}$ days in the chorioallantoic membrane, suggests that they probably entered and left cells at this stage also. The retention of motility in sporozoite-shaped schizonts and their capacity to invade cells may have allowed further development of the schizonts at any time. This would explain the relatively long period of time during which immature and mature large schizonts were seen (Table 1).

Jeffers and Wagenbach (1969, 1970) and Long (1970, 1973) reported hemorrhaging by embryos into the allantoic cavity and death in a portion of the embryos infected with E. tenella. These events occurred $3\frac{1}{2}$ to 4 days post-inoculation and are thought to have resulted primarily from colonies of developing second-generation schizonts. Profuse hemorrhaging, occurring 5 to 6 days after inoculation, was associated with the accumulation of gametocytes and oocysts near blood vessels of the chorioallantoic membrane (Long,

1973). Long (1973) discovered that embryos which did not survive E. tenella infections, had macroscopic lesions caused by second-generation schizonts along the walls of blood vessels. Very slight hemorrhaging was observed in the chorioallantoic membrane of embryos infected with E. dispersa. Microscopic examination of the membranes at the site of the lesion revealed clusters of mature or nearly mature schizonts. Schizonts and gametocytes near the blood vessels accompanied the lesions, but since the hemorrhaging was slight, it was difficult to determine whether infection with the parasite was the primary cause of the bleeding.

The most noticeable change in epithelial cells of the chorioallantoic membrane containing endogenous stages of E. dispersa, was an increase in the size of the host cell. The cytoplasm appeared to be stretched around developing schizonts and gametocytes, and became thicker as the parasite matured. Patton (1965) described an 8- to 10-fold increase in chick fibroblasts infected with schizonts of E. tenella. Fayer and Hammond (1967) discovered that all stages of E. bovis, from sporozoites to large schizonts, in cultured bovine cells were surrounded by a parasitophorous vacuole. Similar observations have been made by Speer et al. (1970) with E. callospermophilii and E. bilamellata, and by DeVos et al. (1972) with E. crandalis. Vacuolization of the host epithelial cell was very pronounced with E. dispersa in the

present study, especially around nearly mature schizonts and gametocytes. However, it is not known whether these vacuoles were associated with the parasitophorous vacuole. Mature large schizonts of E. dispersa were sometimes surrounded by fibrocytic cells. This was the most noticeable cellular response to the presence of the parasite. These large schizonts, along with their merozoites, appeared to degenerate after becoming surrounded by the fibrous tissue.

CONCLUSIONS

In the present study, the sporulated oocysts of Eimeria dispersa from the Bobwhite quail are described, and the endogenous development of this parasite in quail embryos is reported.

The endogenous cycle of E. dispersa was observed to undergo complete development in the chorioallantoic membrane of embryos, with oocysts being formed 8 days after inoculation. In embryos, the life cycle appears to be delayed beyond that observed in adult birds. When embryo-derived oocysts are removed from the allantois, sporulated, and reinoculated into young Bobwhite quail, the prepatent period is also delayed beyond that observed in birds inoculated with oocysts derived from natural sources.

As with naturally infected adults, 2 generations of schizonts appear to be representative of the life cycle in

embryos, with the first-generation schizonts being larger than the second-generation schizonts and containing more merozoites than the second-generation schizonts. The gametocyte stages appear to be similar to those reported for other avian species of Eimeria.

E. dispersa was found to be relatively non-pathogenic in adult Bobwhites, but markedly pathogenic in young Bobwhites 2 weeks of age and younger.

Attempts to infect turkeys, chickens, and Japanese quail with sporulated oocysts of E. dispersa isolated from adult Bobwhite quail were unsuccessful, indicating that E. dispersa may be host specific solely for Bobwhite quail.

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